[CONTRIBUTION FROM RIKER LABORATORIES]

Alkaloids of *Rauwolfia serpentina* Benth. II.[1] The Isolation of Naturally Occurring Py-tetrahydroserpentine (Ajmalicine) and a Contribution Toward its Structure

BY M. W. KLOHS, M. D. DRAPER, F. KELLER, W. MALESH AND F. J. PETRACEK

RECEIVED SEPTEMBER 8, 1953

Ajmalicine has been shown to be identical with py-tetrahydroserpentine. On the basis of chemical data and spectral interpretations the empirical formulas of py-tetrahydroserpentine and serpentine have been revised to $C_{21}H_{24}O_3N_2$ and $C_{21}H_{20}O_3N_2$, respectively, and new structures have been proposed incorporating the grouping $CH_3OOC-C=C-O-$ in ring E.

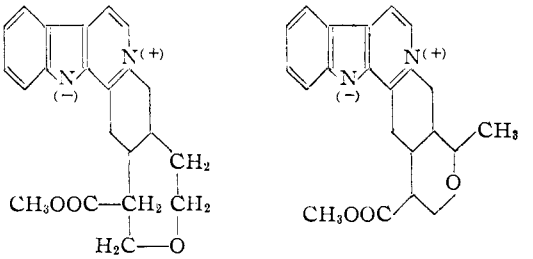
Rauwolfia serpentina Benth (family: *Apocynaceae*) is a short shrub native to India. The plant is described in the early Indian literature as a curative for many common ills. Recently the drug has gained a wide reputation for its hypotensive and sedative properties.

The early chemical investigations of *Rauwolfia serpentina* were quite limited prior to the work of Siddiqui and Siddiqui.[2-5] These investigators succeeded in isolating five crystalline alkaloids which they classified into two groups: the ajmaline group, composed of three white weak bases, ajmaline, ajmalinine and ajmalicine; the serpentine group, composed of two yellow strong bases, serpentine and serpentinine. In a later investigation, with roots grown under different climatic conditions, these same investigators reported the following alkaloids to be present: ajmaline, isoajmaline, neoajmaline, serpentinine and two unidentified alkaloids. Van Itallie and Steenhauer[6-7] in an independent investigation reported the isolation of three alkaloids, two of which are believed to be identical with ajmaline and serpentinine. Recently Chatterjee and Bose,[8] and Muller, *et al.*,[9] have added two more alkaloids to this series with their isolation of rauwolfine and reserpine, respectively.

In the course of an investigation carried out in this Laboratory on the physiologically active principles of *Rauwolfia serpentina*,[1] one of the alkaloids isolated was identified as ajmalicine by a comparison of its physical properties with those reported by Siddiqui and Siddiqui for this alkaloid. These workers succeeded in isolating ajmalicine in quantities sufficient only to report the melting points of the free base, the hydrochloride and picrate derivatives, and to describe the acid color reactions. No further characterization of ajmalicine has since been reported.

The analyses of ajmalicine and three of its derivatives were in excellent agreement for the empirical formula $C_{21}H_{24}O_3N_2$ and showed the presence of one methoxyl group. On comparing the infrared and ultraviolet absorption spectra of ajmalicine with those recently reported by Bader[10-11] for py-tetrahydroserpentine they were found to be identical; the melting points and optical rotations were also in good agreement. Their identity was confirmed by comparing ajmalicine with a sample of py-tetrahydroserpentine obtained by the hydrogenation of serpentine. Ajmalicine has therefore been shown to be py-tetrahydroserpentine.

The first structure for serpentine (I) was proposed by Schlittler and Schwarz[12] as a result of their chemical investigations and degradation of serpentine to alstyrine by selenium dehydrogenation. This structure has since been modified by Bader and Schwarz[10] on the basis of C-methyl determinations making ring E six-membered and incorporating a C-methyl group therein as shown in formula II; thus making serpentine a stereoisomer of an unknown dihydroalstonine. As with alstonine, ring C can be reduced in methanol with platinum and hydrogen yielding the py-tetrahydro derivative.



I

II

The empirical formula postulated for py-tetrahydroserpentine on the basis of our analytical data differs from that previously formulated by Bader and Schwarz[10] by two less hydrogen atoms indicating an additional center of unsaturation in the molecule. Further support for the presence of this additional double bond is gained by a study of the infrared and ultraviolet spectra of py-tetrahydroserpentine which point to the existence of a $CH_3OOC-C=C-O-$ group in ring E. The spectral characteristics of this grouping have been demonstrated in the elegant studies of Bader,[10] and Janot and Goutarel[13] in connection with their work on alstonine and corynantheine, respectively.

The infrared spectrum of py-tetrahydroserpentine (Fig. 1) exhibits a slight shift of the ester carbonyl toward 6μ, which is indicative of an ester

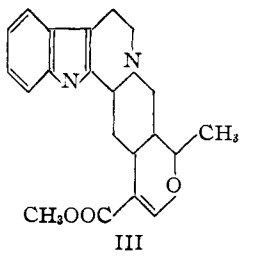
(1) For paper 1 of this series see THIS JOURNAL, **75**, 4867 (1953).
(2) S. Siddiqui and R. H. Siddiqui, *J. Ind. Chem. Soc.*, **8**, 667 (1931).
(3) S. Siddiqui and R. H. Siddiqui, *ibid.*, **9**, 539 (1932).
(4) S. Siddiqui and R. H. Siddiqui, *ibid.*, **12**, 37 (1935).
(5) S. Siddiqui, *ibid.*, **16**, 421 (1939).
(6) L. Van Itallie and A. J. Steenhauer, *Arch. Pharm.*, **270**, 313 (1932).
(7) L. Van Itallie and A. J. Steenhauer, *Pharm. Weekblad*, **69**, 334 (1932).
(8) A. Chatterjee and S. Bose, *Science and Culture*, **17**, 139 (1951).
(9) J. M. Muller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 330 (1952).
(10) F. E. Bader, *Helv. Chim. Acta*, **36**, 215 (1953).
(11) F. E. Bader and H. Schwarz, *ibid.*, **35**, 1594 (1952).
(12) E. Schlittler and H. Schwarz, *ibid.*, **33**, 1463 (1950).
(13) M. M. Janot and R. Goutarel, *Bull. soc. chim.*, **18**, 588 (1951).

carbonyl conjugated with a double bond although the intensity of the band at 6.2μ is greater than that expected for an olefinic bond in this system. It has been shown,[9,12] however, that the presence of an oxygen atom as an enol ether in an α,β-unsaturated ester system such as $CH_3OOC-\underset{|}{C}=\underset{|}{C}-O-$ is accompanied in the infrared spectrum by a shift of the olefinic band to a longer wave length while its intensity is increased to almost equal that of the ester band.

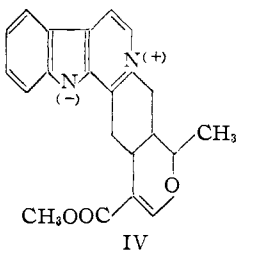
The ultraviolet absorption spectrum of py-tetrahydroserpentine exhibits the two characteristic maxima of a normal α,β-disubstituted indole derivative, at 225 and 280 mμ; the minimum at 246 mμ common to these derivatives is lacking, however, and instead a shoulder is evident in this region resulting in a spectrum essentially the same as that of py-tetrahydroalstonine and corynantheine. This increase of absorption in the region of 250 mμ has been attributed in these latter compounds to the chromophore $CH_3OOC-\underset{|}{C}=\underset{|}{C}-O-$ as a result of spectral studies with model compounds.[10,13]

On subjecting py-tetrahydroserpentine to sodium and butanol reduction a product was obtained which we have designated hexahydroserpentinol. The analyses of the free base and its O-acetyl derivative were in agreement for the empirical formula $C_{20}H_{28}O_2N_2$. The infrared spectra when compared with that of py-tetrahydroserpentine showed the appearance of a hydroxyl band and the absence of the ester carbonyl as well as the intense band at 6.2μ which has been attributed to a conjugated enol ether system. The ultraviolet spectrum now showed a distinct minimum at 246 mμ and was identical to that of hexahydroalstonol.[14] This evidence is compatible with the reduction of the $CH_3OOC-\underset{|}{C}=\underset{|}{C}-O-$ group to $HOC-\underset{|}{C}-\underset{|}{C}-O-$. On lithium aluminum hydride reduction py-tetrahydroserpentine afforded the corresponding alcohol of the composition $C_{20}H_{28}O_2N_2$. The infrared spectrum of this product, tetrahydroserpentinol, exhibited a hydroxyl and an enol ether band and no ester carbonyl. The strong enol ether band in this case has shifted to 6.04μ due to loss of conjugation with the ester.

We now propose, on the basis of this new chemical and spectral evidence, that the empirical formulas for py-tetrahydroserpentine and serpentine be revised to $C_{21}H_{24}O_3N_2$ and $C_{21}H_{20}O_3N_2$, respectively,



III



IV

(14) R. C. Elderfield and A. P. Gray, *J. Org. Chem.*, **16**, 506 (1951).

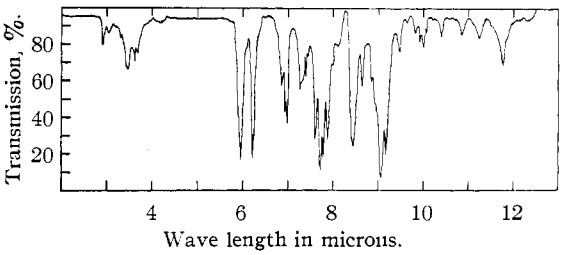


Fig. 1.—Infrared spectra of py-tetrahydroserpentine in chloroform.

and that their structures be modified to incorporate the additional center of unsaturation in ring E, as represented by formulas III and IV.

Serpentine should therefore be considered a stereoisomer of alstonine and not of the unknown dihydroalstonine as has previously been proposed.[10]

Experimental[15]

Extraction of the Roots of *Rauwolfia serpentina*.—Ground, dried roots of *Rauwolfia serpentina* Benth[16] (5 kg.) were extracted with six 14-l. portions of 90% ethanol; the extractions were carried out in a stainless steel tank equipped with stirrer and drain. Each extraction was stirred for eight hours, drained through filter pads into glass stills, and concentrated at 100–120 mm. pressure to a viscous liquid. On completion of the last extract the combined concentrates were evaporated to dryness on the steam table at 100–120 mm. pressure, yielding a tan colored resin (585 g.).

Preliminary Fractionation of the Crude Extract.—A portion of the resin (250 g.) obtained above was powdered with a mortar and pestle and then added with vigorous stirring to one l. of distilled water. After stirring for one-half hour the aqueous extract was decanted off and the remaining tarry material was further extracted with one 1 l. and three 500-ml. portions of water. The dark brown water-insoluble material remaining was dried to a semi-solid mass in a vacuum desiccator (38.0 g.) and designated fraction I. The combined aqueous extracts were then adjusted to *p*H 9.7 with ammonium hydroxide yielding a bright yellow precipitate which was recovered by filtration (fraction II, 24 g.). The mother liquors were extracted five times with chloroform (250-ml. portions); the combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness on the steam table at 100–120 mm. pressure (fraction III, 14.3 g.). The remaining aqueous layer was readjusted to *p*H 11.7 with 6 *N* sodium hydroxide and extracted five times with chloroform (250-ml. portions). The chloroform extracts were combined, dried over sodium sulfate and taken to dryness under vacuum (fraction IV, 2.5 g.).

Fraction III was dissolved as much as possible in a solution of 15% methanol in chloroform (100 ml.). The insoluble residue was removed by decantation and the dark brown solution was chromatographed on an acid-washed alumina column using 15% methanol in chloroform as the developing solvent. The organophilic material in the first four 100-ml. fractions collected, after evaporating to dryness (5 g.), were combined with fraction II above. The isolation of the more hydrophilic alkaloids remaining on the column will be described in a further communication.

The combined fraction II and organophilic alkaloids of fraction III were dissolved in 100 ml. of chloroform and chromatographed on acid-washed alumina using chloroform as the developing solvent; fractions of 150 ml. were collected. Fraction 2 (4.13 g.) and fraction 3 (1.25 g.) were taken to dryness and dissolved separately in 10 ml. of ethyl acetate. Ajmalicine crystallized from both fractions as fine needles.

Ajmalicine.—The crude ajmalicine (0.422 g.) was crystallized several times from methanol yielding stout, colorless needles, m.p. 250° (dec., open tube) 261–263° (no dec., vac.); $[\alpha]^{24}D$ −58.1 ± 2° (*c* 1.10 in $CHCl_3$); −39 ± 4° (*c* 0.25 in methanol). Siddiqui and Siddiqui[2] report a melt-

(15) All melting points are corrected. The melting points have been taken in both open and evacuated capillaries and are so designated.

(16) The plant material used in this investigation was identified by Dr. H. W. Youngken, Mass. College of Pharmacy, Boston, Mass.

ing point of 250–252° dec. The sample showed no melting point depression when admixed with known py-tetrahydroserpentine. The infrared (Fig. 1) and ultraviolet absorption curves of ajmalicine and py-tetrahydroserpentine were identical. For analysis the sample was dried to constant weight at 110° (2 mm.).

Anal. Calcd. for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.87; N, 7.95; $-OCH_3$, 8.82. Found: C, 71.65, 71.46; H, 6.90, 7.06; N, 7.83, 8.16; $-OCH_3$, 8.51, 8.60; no N-CH_3 found.

Ajmalicine Hydrochloride.—Ajmalicine (30 mg.) was dissolved in methanol (10 ml.) and filtered. The solution was concentrated to a volume of 3 ml. on the steam-bath, whereupon 2 drops of concentrated hydrochloric acid was added. On cooling, ajmalicine hydrochloride crystallized as white, irregular sheafs, m.p. 265–268° (dec. open), 292–293° (vac.). Siddiqui and Siddiqui[2] report a melting point of 260–263° dec. For analysis the sample was dried to constant weight at 140° (2 mm.).

Anal. Calcd. for $C_{21}H_{24}O_3N_2 \cdot HCl$: C, 64.85; H, 6.48; Cl, 9.10. Found: C, 64.62; H, 6.46; Cl, 9.29.

Ajmalicine Hydrobromide.—Ajmalicine hydrobromide was made in the same manner as described for ajmalicine hydrochloride using concentrated hydrobromic acid. The product crystallized as diamond-shaped platelets, m.p. 295–296° (vac.). For analysis the sample was dried to constant weight at 100° (2 mm.).

Anal. Calcd. for $C_{21}H_{24}O_3N_2 \cdot HBr$: C, 58.20; H, 5.81; N, 6.46; Br, 18.44. Found: C, 58.19; H, 5.89; N, 6.50; Br, 18.52.

Ajmalicine Hydroiodide.—Ajmalicine hydroiodide was prepared in the same manner as described for ajmalicine hydrochloride using concentrated hydroiodic acid. On crystallization from dilute methanol, triangular faced prisms were obtained, m.p. 291–293° (vac.). For analysis the sample was dried to constant weight at 100° (2 mm.).

Anal. Calcd. for $C_{21}H_{24}O_3N_2 \cdot HI$: C, 52.51; H, 5.43; N, 5.83; I, 26.42. Found: C, 52.32; H, 5.58; N, 5.89; I, 26.33.

Ajmalicine Picrate.—Ajmalicine (50 mg.) was dissolved in 5% aqueous acetic acid (6 ml.). Picric acid (1% aqueous solution) was added dropwise until no further precipitation occurred. The yellow precipitate was recovered and dried under vacuum. Crystallization was effected by dissolving in hot absolute ethanol and allowing to stand; needles, m.p. 212–213° (open or vac.). Siddiqui and Siddiqui[2] report a melting point of 212–215°.

Py-tetrahydroserpentine.—Serpentine (1.1 g.) was hydrogenated according to the procedure of Bader and Schwarz.[11] The product obtained was dissolved in $CHCl_3$ (50 ml.) and then chromatographed on an acid-washed alumina column. Py-tetrahydroserpentine (0.8 g.) was eluted with chloroform while the unreacted serpentine remained near the top of the column. Py-tetrahydroserpentine crystallized as stout, colorless needles from methanol, m.p. 250–252° (dec., open tube). Bader and Schwarz reported a melting point of 250°.

The infrared spectrum (Fig. 1) was also taken in a nujol mull for comparison with that of hexahydroserpentinol due to the latter compound's insolubility in chloroform, and showed a strong conjugated ester carbonyl band at 5.95 μ and a more intense band at 6.25 μ attributed to the conjugated enol ether absorption. The ultraviolet spectrum in ethanol was the same as that reported for tetrahydroalstonine[14]: λ_{max} (log ϵ) 280 (3.91), 225 (4.68). For analysis the sample was dried to constant weight at 66° (2 mm.).

Anal. Calcd. for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.87. Found: C, 71.68; H, 7.19.

Hexahydroserpentinol[17].—Py-tetrahydroserpentine (340 mg.) was reduced with sodium and butanol by the procedure employed by Leonard and Elderfield in the reduction of tetrahydroalstonine.[18]

A crystalline product (195 mg.) separated upon the removal of the butanol by steam distillation. The material was crystallized from absolute ethanol, m.p. 248–250° (open, extensive dec.) 333–334° (vac.); $[\alpha]^{24}D$ $-95 \pm 2°$ (c 0.92 in pyridine); the infrared spectrum (nujol) showed the hydroxyl band at 3.1 μ, and had no absorption in the region of 5.0–6.6 μ. The ultraviolet spectrum in ethanol was the same as that reported for hexahydroalstonol[14]: λ_{max} (log ϵ) 280 (3.94), 225 (4.58); λ_{min} (log ϵ) 246 (3.06). For analysis the sample was dried to constant weight at 130° (2 mm.).

Anal. Calcd. for $C_{20}H_{26}O_2N_2$: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.62; H, 8.04; N, 8.56.

O-Acetylhexahydroserpentinol.—Hexahydroserpentinol (100 mg.) was dissolved in pyridine (15 ml.) and acetic anhydride (1 ml.) was added. The reaction mixture was allowed to stand 24 hours at room temperature. At the end of this time the solution was evaporated *in vacuo* to dryness. The residue was taken up in dilute acetic acid, made basic with ammonium hydroxide and extracted with chloroform. The chloroform was removed *in vacuo* and the residue crystallized from dilute ethanol as needles, m.p. 164° (vac.) with preliminary sintering. The infrared spectrum showed ester carbonyl absorption at 5.8 μ. For analysis the sample was dried to constant weight at 80–90° (2 mm.).

Anal. Calcd. for $C_{22}H_{28}O_3N_2$: C, 71.71; H, 7.66. Found: C, 71.74; H, 7.72.

Tetrahydroserpentinol.[17]—Py-tetrahydroserpentine (200 mg.) was reduced with lithium aluminum hydride by the procedure employed by Elderfield and Gray in the reduction of tetrahydroalstonine.[14] The product obtained (150 mg.) crystallized from dilute ethanol as stubby needles, m.p. 260° (vac.) with preliminary sintering; m.p. 244° with extensive decomposition (open); $[\alpha]^{24}D$ $-136 \pm 2°$ (c 1.00 in pyridine). The infrared spectrum (nujol) showed the hydroxyl band at 3.1 μ and the enol ether band at 6.04 μ. The ultraviolet absorption spectrum in ethanol was identical to that reported for tetrahydroalstonol[14]: λ_{max} (log ϵ) 280 (3.88), 225 (4.50); λ_{min} (log ϵ) 246 (3.14). For analysis the sample was dried to constant weight at 110° (2 mm.).

Anal. Calcd. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 74.09; H, 7.53; N, 8.53.

Acknowledgments.—The authors are indebted to Dr. Adalbert Elek for the microanalyses, and to M. Robinson and C. H. Stimmel for the optical rotations, infrared and ultraviolet absorption spectra.

LOS ANGELES, CALIFORNIA

(17) The designation tetrahydroserpentinol has previously been assigned (H. Schwarz and E. Schlittler, *Helv. Chim. Acta*, **34**, 629 (1951)) to the product obtained by the sodium and butanol reduction of serpentine nitrate. In view of the present findings this compound should be named hexahydroserpentinol.

(18) N. J. Leonard and R. C. Elderfield, *J. Org. Chem.*, **7**, 556 (1942).